Specific Topics

- Basal transcription by RNA polymerase II
- Sequence-specific DNA-binding factors
- How might enhancers work?
- Chromatin structure – Introduction
- Covalent modification of histones
- Chromatin remodeling factors
- Chromatin assembly
DNA Regulatory Elements (cis elements) for Transcription of Protein-coding Genes by RNA Polymerase II

Enhancers
Core Promoters
Proximal Promoters
Boundary (Insulator) Elements
Many Factors Affect the Regulation of Transcription by RNA Polymerase II

Ubiquitinylating Enzymes  
CpG Methylation  
HDAC Complexes  
p300/CBP  
ARC/TRAP/DRIP/SMCC/NAT/Mediator/SRB/CRSP complex  
Boundary Elements  
Heterochromatin  
ATP-utilizing Chromatin Remodeling Factors  
CH$_3$  
Kinases  
Polycomb Group Proteins  
Protein Acetyltransferases  
Promoter- and Enhancer-binding Factors  
SRC/p160 Proteins  
Basal/General Transcriptional Machinery  
Dr1-Drap1/NC2 Mot1 NOT proteins  
Srb10-Srb11  
Protein Methyltransferases  
RNAi Machinery  
Boundaries
Sequence-specific DNA-binding Transcription Factors Are the Apex at the Interface of Genetic Regulatory Information and the Inverted Cone of Other Transcription Factors
Sequence-specific Transcription Factors Are Modular
Chromatin Is an Integral Component of Transcription
A TENTATIVE MODEL FOR GENE ACTIVATION

- Activated State
  - True Activation
    - (or, “unrepressed state”; = Naked DNA in vitro)
- Derepressed State
- Inactive Ground State

GENE ACTIVITY
Sequence-specific Factors Typically Bind in Clusters

Enhancer

Proximal Promoter
Nuclear Receptors Are an Interesting Family of Sequence-specific DNA-binding Transcription Factors

- Sequence-specific DNA-binding proteins
- Upon binding of their cognate ligands (agonists), they activate transcription.
- Thus, nuclear receptors function as both the receptor for the signals (agonists) as well as sequence-specific DNA-binding transcriptional activators.
- Inactivated by antagonists, which are ligands that resemble the agonists, but block activation functions.
- Examples include estrogen receptor, androgen receptor, glucocorticoid receptor, vitamin D receptor, thyroid hormone receptor.
Sequence-specific Factors Appear to Work by Recruitment of Coregulators

- TAF subunits of TFIID
- p300 and CBP
- Mediator (SRB complex, TRAP, DRIP, ARC, CRSP, SMCC, NAT)
- ATP-dependent chromatin remodeling factors
- Histone-modifying enzymes
How Do the Sequence-specific Factors Work?

* Sequence-specific factors probably activate or repress transcription via direct and indirect mechanisms of communication with the basal/general factors that carry out the transcription process.

• There can be direct interactions between the sequence-specific regulators and the TAF subunits of TFIID.

• The sequence-specific factors can interact with coregulators, such as p300/CBP and Mediator (also known as SRB complex, TRAP, DRIP, ARC, CRSP, SMCC, NAT), to recruit the basal transcription machinery to the core promoter. p300/CBP also has acetyltransferase activity and can acetylate histones and other proteins.

• The sequence-specific factors can recruit ATP-dependent chromatin remodeling factors to the chromatin template. These factors catalyze the movement of nucleosomes and thus facilitate the transcription process.

• The sequence-specific factors can recruit histone-modifying enzyme complexes to the chromatin template. The modification of histones could affect the biochemical properties of chromatin (such as histone-DNA interactions or chromatin folding) as well as act as signals for the binding of other factors to the chromatin template.

* Thus, in a general sense, the sequence-specific factors act to recruit the basal transcriptional machinery to the core promoter as well as to recruit chromatin remodeling and modifying enzymes that facilitate the function of the transcription factors in the milieu of chromatin.
DNase I Footprinting Analysis of Sequence-specific DNA-binding Proteins

Partial DNase I digestion gives single-stranded nicks

No Factor (Control/Reference) + Sequence-specific Factor

Electrophoresis

Autoradiography of Labelled DNA Fragments
Mutation of the DPE Reduces Binding of TFIIID
Gel Mobility Shift Analysis of Sequence-specific DNA-binding Proteins

Sequence-specific Factor

Labelled double-stranded DNA fragment

No Factor (Control/Reference) + Sequence-specific Factor + Sequence-specific Factor + Antibody

"Supershift"

Autoradiography of Labelled DNA Fragments

Electrophoresis
Sequence-specific DNA Affinity Chromatography

Protein Fraction
+ Nonspecific Competitor DNA

Sequence-specific Proteins

Nonspecific Proteins

Specific DNA Recognition Sites

Sequence-specific DNA Affinity Resin

Sequence-specific DNA Affinity Resin

Wash Resin and Elute Purified Sequence-specific DNA-binding Protein
Chromatin Immunoprecipitation (ChIP) Analysis

1. Formaldehyde fixation of chromatin in living cells
2. Sonication
3. Chromatin purification and immunoprecipitation
4. Reversal (hydrolysis) of crosslinks
5. Analysis of immunopurified DNA sequences (typically, by PCR)

Sequence-specific DNA-binding Transcription Factors (RNA Pol II)

- Modular Structure
  - Sequence-specific DNA-binding Modules
  - Transcriptional Activation/Repression Modules
  - Regulatory Modules (inter- or intramolecular)
  - Multimerization Modules (homo- and heterotypic interactions)
- Regulate Transcription via Recruitment of Coactivators and Corepressors
- Chromatin Is an Integral Component in the Function of Sequence-specific Factors
- Sequence-specific Factors Can Be Regulated by Post-translational Modifications
- Sequence-specific Factors Are Often Members of Multiprotein Families
- Recognition Sites for Sequence-specific Factors Tend to Be Located in Clusters
- Sequence-specific Factors Typically Bind to DNA with Relatively Low Specificity
- Sequence-specific Factors Can Affect Transcription Initiation and/or Elongation
- Some Factors Are Commonly Found in Proximal Promoter Regions
- Sequence-specific Factors Bind to Boundary/Insulator Elements
- Some Sequence-specific Factors Can Bend DNA
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How Do Enhancers Work?

Transcriptional Enhancers

Promoters

Boundary or Insulator Elements
Enhancer-Promoter Specificity vs. Insulator/Boundary Function

Specific Enhancer-Promoter Interactions

Insulation of Enhancer Function by Boundary Elements
What happens when you see an increase in transcription in a population of cells?

**Probability (On or Off) Model**
Enhancers increase the probability of transcription but not the amount of transcription in a cell.

**Progressive Response Model**
Enhancers uniformly increase the amount of transcription in a cell in a continuous manner.
A Facilitated Tracking Model for Enhancer Function

1. Sequence-specific Activators Bind to the Enhancer

2. A Small Loop Is Formed as Activators and Coactivators Begin Tracking

3. Enhancer-Promoter Connection Is Established
Many Factors Affect the Regulation of Transcription by RNA Polymerase II